Using Guanine Rich Aptamer Strands to Increase Cellular Internalization of DNA Nanostructures

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Abstract:

DNA, while best known for its coding abilities, can also be used as a structural material to form nanostructures. DNA nanostructures can be designed with very specific chemistry and structure size and shape, and have the potential to become very effective delivery vehicles for biomolecules and therapeutic agents. Past research has shown that guanine rich DNA strands increase cellular internalization of nanoparticles, possibly due to the secondary G-quadruplex structures that they form in the presence of potassium. The purpose of this research is to study the use of guanine rich aptamer strands on cellular internalization of DNA nanostructures. DNA origami was used to create DNA nanostructures, and guanine rich aptamer strands were annealed to the structures. AFM was used to verify the formation of the structures, circular dichroism was used to verify the formation of the G-quadruplexes, and gel electrophoresis was used to verify the attachment of the aptamer strands to the DNA nanostructures. Flow cytometry was used to quantify cellular internalization of the DNA nanostructures in HPV cancer cell lines. The results of this research show that the use of guanine rich aptamer strands on DNA nanostructures increase cellular internalization of DNA nanostructures, further demonstrating the potential for DNA nanostructures to effectively be used as delivery vehicles for biomolecules and therapeutic agents.

Introduction:

DNA is most commonly known for its role in genetic coding, but it can also be used as a building material to create DNA nanostructures. Utilizing the complementary base pair binding of DNA and self-assembling formation, DNA nanostructures can be engineered to form structures with very specific sizes, shapes, and surface chemistries. DNA nanostructures are a family of cell penetrating peptide, better know as CPPs, and have become one of the more effective delivery vehicles of therapeutic molecules across cell membranes. CPPs are less cytotoxic than many other common alternative methods, such as viral-based vectors[1], and their positive net charge allows them to bond to larger biomolecules, such as nucleic acids and chemotherapeutic drugs, and transport them across plasma membranes[1]. While there is still more to be learned about their long term effects, current studies have been able to show that CPPs are able to enter cells, primarily through endocytosis, and release therapeutic molecules into the cytosol[1]. Because CPPs, specifically DNA nanostructures, can be engineered to have such specific structure and chemistry, they have the ability to become a universal delivery vehicle for therapeutic molecules.

DNA nanostructures can be formed using a variety of different methods, but one of the most common methods is using DNA origami. DNA origami involves using a single “scaffold” strand, normally an M13 viral genomic DNA strand, several thousand bases long, that is folded into a determined structure using smaller “staple” strands with complementary bases to specific regions of the scaffold strand. The staple strands are used to fold the scaffold strand and hold it in place in its final form. This method of building DNA nanostructures has successfully been used to build a multitude of different

structures, up to thousands of bases long, and is what controls the size and shape of the final nanostructure.

A study done by Suguna P. Narayan et al. investigated the cellular uptake of nucleic acids with varying concentrations of the four bases, adenine, thymine, cytosine, and guanine[4]. The study found that cells internalized nucleic acids that were rich in guanine at higher levels as compared to the strands rich in any of the three bases. One explanation of this is that guanine rich nucleotide strands have been shown to form G-quadruplex secondary structures in the presence of potassium.

G-quadruplexes can be formed in a variety of different ways. A study done by Tomas Simonsson characterized the different folding patterns that can result in g-quadruplex, as well as documented the addition and repetition of different bases and how it affected the final g-quadruplex structure[3]. Based off of Simonsson's work, the two main structural components of g-quadruplexes are the four strands that make up the tetrad structure and the loops caused from folding of the nucleic acid strands at the top and bottom of the structure[3]. Within the four main strands, the guanine bases form planar structures that stack within the core of the G-quadruplex[3]. G-quadruplexes have found to be naturally occurring in a variety of places, including telomeres, promoter regions of different oncogenes, and upstream of the insulin gene, as well as having been found to bind to various proteins[3].

A 2014 study investigated the use of G-quadruplexes attached to gold nanoparticles with the goal of increasing cellular uptake of the gold nanoparticles[2]. The experiment tested a variety of loading densities of the G-quadruplexes onto the gold nanoparticles, and found that increasing the density of G-quadruplexes on the gold

nanoparticles lead to an increased cellular uptake of the gold nanoparticles of up to 20%[2].

While there have been studies showing that cells uptake guanine rich DNA strands at a higher rate than other nucleotide rich strands, and that increased G-quadruplex density on nanoparticles increased cellular internalization, very little has been studied about the effect of guanine rich aptamer strands to increase cellular internalization of DNA nanostructures. The goal of this study is to determine if guanine rich aptamer strands can help increase cellular internalization of DNA nanostructures, in hopes of ultimately making DNA nanostructures more effective delivery vehicles for biomolecules and therapeutic agents.

Methods:

DNA origami was used to form 16 Helix Bundle (16HB) DNA nanostructures out of p7560 scaffold strands, and TEM was used to image the structures and confirm their formation. Two guanine rich aptamer strands (GGT and GQuad) were used to test attachment to 16HB nanostructures and for G-quadruplex formation. Circular dichroism was used to confirm the formation of G-quadruplexes of both strands when put in a 150mM potassium solutions. A 1.5% agarose gel electrophoresis was used to confirm the attachment of the strands to the 16HB nanostructures.

Small DNA tetramer nanostructures were formed using DNA origami. A guanine rich aptamer strand was annealed to one vertex of each tetramer, and Cy5 fluorescent dye was annealed to the other three vertices. 1.5% agarose gel electrophoresis was used to confirm the attachment of the aptamer strand and Cy5. Cell internalization

studies were performed on four unique HPV cancer cell lines, and flow cytometry was used to quantify the internalization of the structures.

Results:

Formation of 16HB DNA Nanostructures

TEM was used to image the successful formation of 16HB nanostructures at 70,000 time magnification. Several structures appeared to form dimers as seen in Figure 1.

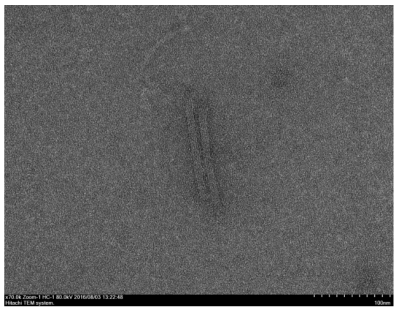

Figure 1

G-Quadruplex Formation

Circular dichroism was used to verify the formation of G-quadruplexes in the presence of potassium. Figure 2 shows both the GGT and G-quad aptamer strands in 150mM potassium buffer. The wavelength plots show that there is a difference in light absorption between the guanine rich strands and the control strand, signifying the likely formation of a G-quadruplex.

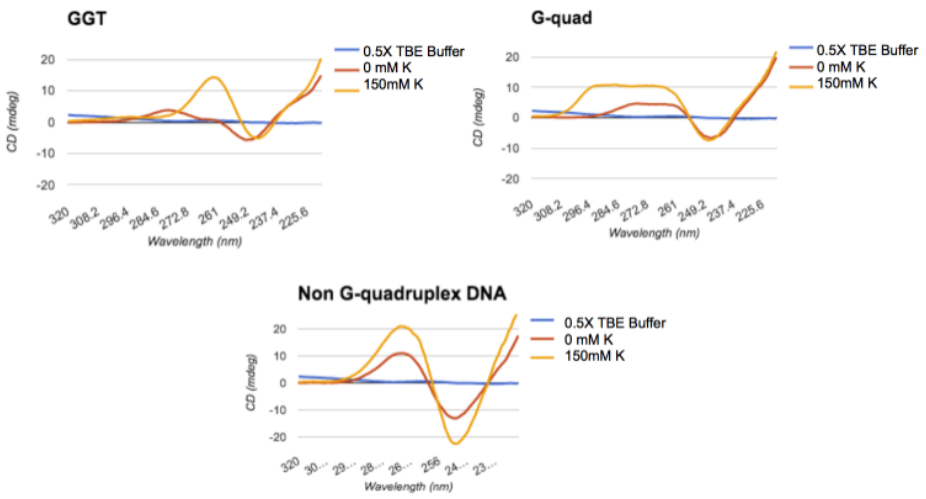

Figure 2

Aptamer Strand Attachment

Gel electrophoresis was used to verify the attachment of aptamer strands to 16HB nanostructures. Figure 3 shows the addition of each of the aptamer strands to the 16HB nanostructures both pre-formed in a potassium solution, and with the addition of a potassium solution after attachment. The gel shows that the aptamer strands did attach to the nanostructures, but do not show a difference between the addition of the pre and post formed g-quadruplex structures.

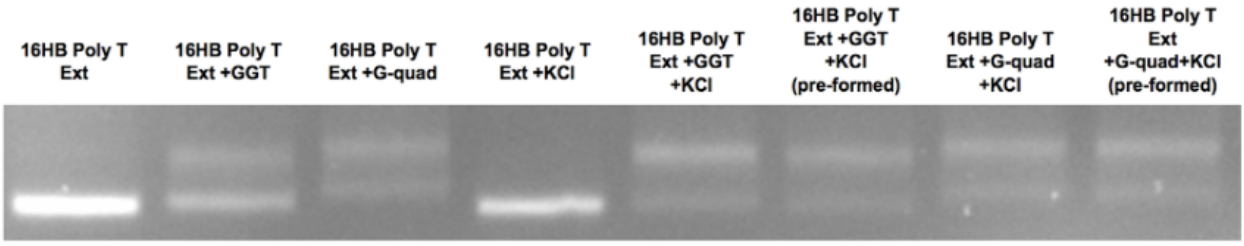

Figure 3

Cell Internalization

DNA tetramer structures with three aptamer strand and one Cy5 dye strand attachments were introduced into HPV cancer cells and flow cytometry was used to quantify their internalization into the cells. Figure 4 shows that in four out of the five cell lines, the DNA tetramers with guanine rich aptamer strands had the highest level of

internalization when compared to just the Cy5 strands, tetramers with no aptamer strands, and tetramers with aptamer strands that were not rich in guanine.

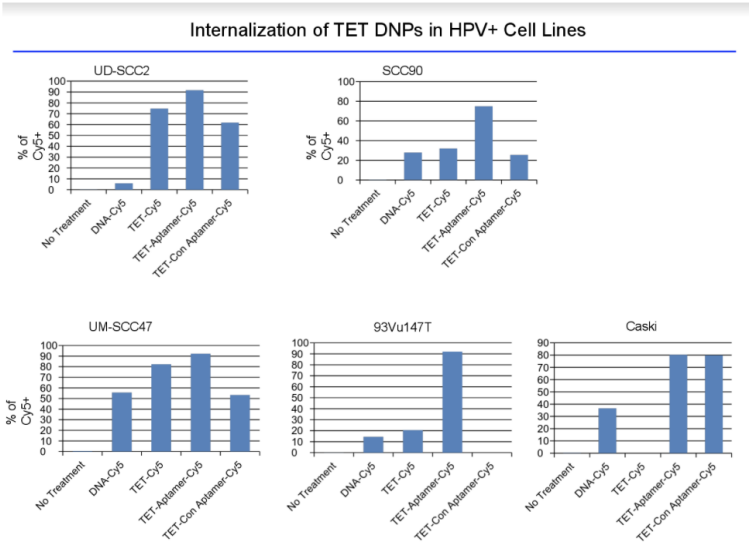

Figure 4

Discussion:

As a whole, this data suggests that guanine rich aptamer strands are effective at increasing cellular internalization of DNA nanostructures. The circular dichroism results confirm that the specific aptamer strands are capable of forming G-quadruplexes, which are likely a reason for the increased cellular internalization. The results also show that the order of formation and attachment of the G-quadruplexes have little effect on the overall joint structure. This work shows that guanine rich aptamer strands, and possibly G-quadruplexes could play a key role in increasing cellular internalization of DNA nanostructures, and additional research can be done to understand their full potential.

Future Work:

The next step in the project would be to optimize the size and shape of the DNA nanostructures for maximum cellular internalization, as well as optimize the number of Cy5 and guanine rich aptamer attachment strands. After nanostructure optimization, *in*

vivo studies would need to be performed to test the effect of DNA nanostructures on a larger scale, and successful results could lead to DNA nanostructures becoming increasingly used as a biomolecules and therapeutic agent delivery vehicle.

References:

1. *Dana Maria Copolovici, K. L., Elo Eriste, and U¨lo Langel (2014). "Cell-Penetrating Peptides: Design, Synthesis, and Applications." ACS Nano 8(3): 1972-1994.*

2. *Duncan Hieu M. Dam, R. C. L., and Teri W. Odom (2014). "Improved in Vitro Efficacy of Gold Nanoconstructs by Increased Loading of G- quadruplex Aptamer." Nano Letters(14): 2843-2848.*

3. *Simonsson, T. (2001). "G-Quadruplex DNA Structures – Variations on a Theme." Biol. Chem. 382: 621-628.*

4. *Suguna P. Narayan, C. H. J. C., Liangliang Hao, Colin M. Calabrese, Evelyn Auyeung, Chuan Zhang, Olga J. G. M. Goor, and Chad A. Mirkin (2015). "The Sequence-Specific Cellular Uptake of Spherical Nucleic Acid Nanoparticle Conjugates." Small: 1-10.*

5. *Yuta Sannohe, M. E., Yousuke Katsuda, Kumi Hidaka, and Hiroshi Sugiyama (2010). "Visualization of Dynamic Conformational Switching of the G-Quadruplex in a DNA Nanostructure." Journal of American Chemical Society 132: 16311-16313.*